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PATENT & TRADEMARK OFFICE
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)

Kenichi HIGASHIYAMA et al.)

Application No.: 09/254,152)

Filed: February 26, 1999)

For: PROCESS FOR PRODUCING)
UNSATURATED FATTY ACID-)
CONTAINING OILS)

Group Art Unit: 1617

Examiner: Shengjun Wang

Confirmation No.: 6530

DECLARATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Kengo Akimoto, hereby declare:

1. I am one of the inventors of the above-cited application.
2. My Curriculum Vitae is attached hereto as Appendix A.
3. The experiments described herein were performed either by me or under my direct supervision and control.
4. The inventive feature of the above-mentioned patent application is to obtain the microbial oil containing poly-unsaturated fatty acids with less 24,25-methylenecholesterol- 5-en-3beta-ol content obtainable by fermentation of strains belonging to Mortierella subspecies of Mortierella species in a fermentation medium containing nitrogen source derived from soybean.
5. The above-mentioned strains accumulate significant amount of oil (triglycerides) containing poly-unsaturated fatty acids as their constituting fatty acids, which is obtained by extraction with hexane. They produce 24,25-methylenecholesterol- 5-en-3beta-ol (Component A) as well as desmosterol (Component B)

simultaneously, which also exist in the oil extracted. We have to reduce the amount of Component A as much as possible, for it is an unfavorable component of little to none eating habit whereas we have no worry about Component B, which is a component in human breast milk and thus within our eating habit. It is therefore our inventive feature to make the proportional content of Component B higher compared with A to achieve the reduction of Component A, and surprisingly, we have achieved our goal by fermenting the strains in the medium containing nitrogen source derived from soybean.

6. Component A and Component B are both sterols naturally existing in the microbial oil (triglycerides) as unsaponified matter. Sterols may be reduced to a certain amount by conventional oil refining process (degumming, deacidification, bleaching, and deodorization), which is generally applied to edible plant oil/fat production. A lot of reports on the sterol content during the refining process may be obtained, and one of which is shown for the sterol content(mg/g) in soybean oil in Table 1 as an example.

Table 1 Sterol content after the conventional oil refining process				
Process	Process I		Process II	
	Free-type	Ester-type	Free-type	Ester-type
Degumming	3.1	0.6	3.4	0.6
Deacidification	3.0	0.6	3.0	0.6
Bleaching	1.8	0.5	2.0	0.6
Deodorization	1.8	0.5	1.6	0.6

7. Sterols are classified in two types; free-type and ester-type and free-type sterols are the ones removed during the conventional oil refining process and content of ester-type sterols remain unchanged as shown in Table 1. And even free-type sterols may not be removed completely during the refining process. It is therefore important to reduce the sterol content of Component A in the extracted oil before refining process considering the incompleteness of removal of sterol content by refining process.
8. Next the model chart may explain about the concept of sterol content and its reduction.

Prior art:

Extracted oil

Ester-type Component A	Ester-type Component B
Free-type Component A	Free-type Component B

Refining
process →

Refined oil

Ester-type Component A	Ester-type Component B
Free-type Component A	Free type Component B

Present Invention:

Extracted oil

Ester-type Component A	Ester-type Component B
Free-type Component A	Free-type Component B

Refining
process →

Refined oil

Ester-type Component A	Ester-type Component B
Free-type Component A	Free type Component B

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9. The extracted oils naturally contain Ester-type Component A, Free-type Component A, Ester-type Component B, and Free-type Component B, and ester-type sterols remain unchanged during the refining process whereas free-type sterols may not be removed by refining process, either.
10. The refined oil consequently contains Ester-type Component A and Ester-type Component B, both unchanged, and Free-type Component A and Free-type Component B, both exist as certain amount of content although reduced by refining process. In the end in order to obtain the oil with less Component A, which we have little eating habit with, it is important to obtain the oil with more content of Component B, and we have achieved the goal by fermenting the strains belonging to Mortierella subspecies of Mortierella species in a fermentation medium containing nitrogen source derived from soybean.
11. I have read the Official Action dated April 9, 2003. I understand that claims in the instant application have been rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Shinmen et al in view of both Shimizu et al and Barclay. On page 3, the Official Action states:

Therefore, it would have been prima facie obvious to a person of ordinary skill in the art, at the time the claimed invention was made, to modify the unsaturated fatty acid-containing oil of Shinmen et al. by removing the biologically unknown compound, i.e., 24,25-methylenecholest-5-en-3 β -ol and employ the modified oil in food

products such as baby food and animal food or in nutritive dietary supplement.

12. This assertion is in error. As shown *supra*, the 24,25-methylenecholest-5-en-3 β -ol cannot be removed using standard refining processes to produce an arachidonic acid-containing oil in accordance with the claimed invention.

The instant invention thus overcomes the problem in the art of how to reduce the 24,25-methylenecholest-5-en-3 β -ol content in products such as baby food, animal food and nutritive dietary supplements.

13. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

December 8, 2003
DATE

Kengo Akimoto
Kengo Akimoto